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Title: ADAMTS13 gene variants and function in women with preeclampsia: a population- based nested case- control study from the HUNT Study

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Abstract

Introduction

Known genetic variants with reference to preeclampsia only explain a proportion of the heritable contribution to the development of this condition. The association between preeclampsia and the risk of cardiovascular disease later in life has encouraged the study of genetic variants important in thrombosis and vascular inflammation also in relation to preeclampsia. The von Willebrand factor-cleaving protease, ADAMTS13, plays an important role in micro vascular thrombosis, and partial deficiencies of this enzyme have been observed in association with cardiovascular disease and preeclampsia. However, it remains unknown whether decreased ADAMTS13 levels represent a cause or an effect of the event in placental and cardiovascular disease.

Methods

We studied the distribution of three functional genetic variants of *ADAMTS13*, c.1852C>G (rs28647808), c.4143_4144dupA (rs387906343), and c.3178C>T (rs142572218) in women with preeclampsia and their controls in a nested case-control study from the second Nord-Trøndelag Health Study (HUNT2). We also studied the association between ADAMTS13 activity and preeclampsia, in serum samples procured unrelated in time of the preeclamptic pregnancy.

Results

No differences were observed in genotype, allele or haplotype frequencies of the different *ADAMTS13* variants when comparing cases and controls, and no association to preeclampsia was found with lower levels of ADAMTS13 activity.

Conclusion:

Our findings indicate that *ADAMTS13* variants and ADAMTS13 activity do not contribute to an increased risk of preeclampsia in the general population.

Abbreviations

ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin type 1 motifs, member 13; TTP, thrombotic thrombocytopenic purpura; HUNT, Nord-Trøndelag Health Study;

Keywords

ADAMTS13, mutations, preeclampsia, case-control, the HUNT study

Introduction

Preeclampsia is a major contributor to maternal and perinatal morbidity and mortality, affecting 2-8% of pregnancies worldwide[1]. It is clinically characterized by new-onset hypertension and proteinuria in the latter half of pregnancy. In its most severe forms it bears resemblance with thrombotic microangiopathies, with fragmentation of erythrocytes and consumptive thrombocytopenia[2]. The aetiology of preeclampsia is multifactorial and complex, including genetic as well as environmental risk factors, some of them shared with cardiovascular disorders [1]. Essential in the pathogenesis of preeclampsia is impaired trophoblast invasion and spiral artery remodelling, resulting in abnormal implantation and placental hypo-perfusion. Cytokines and reactive metabolites released from the ischaemic placenta are then believed to be responsible for the hypertension and protein leakage through endothelial cell activation, inflammation and clotting activation [1].

ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motifs, member 13) plays an important role in microvascular thrombosis regulation by cleaving von Willebrand factor (VWF) multimers under shear conditions. A constitutional severe deficiency of this metalloprotease (less than 10% of normal activity) through mutations in the *ADAMTS13* gene is the cause of hereditary thrombotic thrombocytopenic purpura (TTP), another thrombotic microangiopathy [3, 4]. Platelet rich thrombi in the microcirculation with different degrees of end organ ischaemia are caused by ultra-large VWF multimers in the circulation in these patients. We and others have previously shown that hereditary TTP in women in reproductive age is encumbered with a high frequency of pregnancy complications and adverse foetal outcomes [5-8]. Pregnancy is considered a trigger of endothelial activation and thrombotic microangiopathy in these patients. Treatment throughout the pregnancy with prophylactic plasma infusions, elevating the levels of ADAMTS13, has proven successful in preventing placental ischaemia and adverse pregnancy outcomes in these patients [8], suggesting a direct role of ADAMTS13 in the pathogenesis. Whether also partial deficiencies of ADAMTS13 play a role in the development of preeclampsia is not established. A case-control study found an association between low ADAMTS13 levels and preeclampsia [9], however, from this study it remains unclear whether low ADAMTS13 activity is a risk factor or a marker of disease. Similar observations of mild ADAMTS13 deficiencies have been made for cardiovascular disease and ischaemic stroke, conditions that share a pathophysiology of endothelial cell dysfunction, inflammatory response and vascular reactivity with preeclampsia [10-14].

In view of this, we aimed to investigate the association of three functional *ADAMTS13* variants with reference to preeclampsia in a population-based nested case-control study. We also studied the association between ADAMTS13 activity and preeclampsia, hypothesizing an increased risk for preeclampsia with lower ADAMTS13 activity.

Methods

Study population

The women in this study were recruited from the second survey of the Nord-Trøndelag Health Study (HUNT2), a large population-based health survey conducted in Nord-Trøndelag County between

August 1995 and June 1997. All residents aged 20 years or older (N= 94194) were invited, and 66140 (71.2% of all eligible) participated in the study. This multi-purpose survey includes standardized measurements of blood pressure, height and weight and a comprehensive questionnaire including questions on socio-economic factors, family and personal history of cardiovascular disease, cerebrovascular disease, diabetes and hypertension, births, medication use and smoking habits. For 98.7% of the participants it also includes a non-fasting blood serum sample and DNA extracted from a blood clot or an EDTA-blood sample. The population of Nord-Trøndelag is considered stable and homogeneous with a net migration rate of 0.3% per year and less than 3% non-Caucasians, and therefore suitable for genetic epidemiological studies. Further details regarding the health survey are described elsewhere [15].

Cases of preeclampsia in the present study were collected from a previous study, which investigated the validity of the preeclampsia diagnosis in the Medical Birth Registry of Norway (MBRN) in a cohort of women with preeclampsia and concomitant participation in the HUNT2 survey [16]. An 11-digit unique personal identification number given every individual in Norway allows a secure linkage between health registries. The diagnostic criteria for preeclampsia used were an increase in blood pressure to at least 140 mmHg systolic or 90 mmHg diastolic once after 20 weeks' gestation (or an increase in diastolic blood pressure of ≥15 mmHg from the level measured before the 20th gestational week) *and* urine protein excretion of ≥0.3 g/24h or ≥1+ on dip-stick once after 20 weeks' gestation. The diagnosis of preeclampsia was confirmed in 853 out of 966 pregnancies under study [16], and a random sample of 500 of the 853 women constitutes the cases in the present study.

As controls 500 women from the HUNT2 survey who had given birth and who were not included as cases in the first study [16] were selected. There was no matching by any variables between cases and controls. Linkage of data between the HUNT2 and MBRN was then performed for the 1000 subjects in the present study. The selection of cases and controls from the HUNT2 survey is described in figures A.1a and A.1b in Appendix A.

Signed informed consent was obtained from all participating subjects in the HUNT2 Survey, and the present study was approved by the Regional Committee for Medical Research Ethics (REC Central approval 2010/558).

ADAMTS13 variants

The gene encoding ADAMTS13 is localized on chromosome 9q, and more than 150 different mutations in this gene have been reported in association with severe ADAMTS13 deficiency in hereditary TTP. Most of the reported mutations are limited to individual families, with two exceptions: 1) Mutation c.4143_4144dupA, where the insertion of a single base (adenine, A) at position 4143_4144 results in a frameshift and loss of 49 amino acids is one of the most frequently reported *ADAMTS13* mutations in Caucasians [17-23] and therefore included in this study. 2) Mutation c.3178C>T (p.Arg1060Trp) in exon 24 is also frequent and associated with late-onset and pregnancy related TTP, and is therefore of special interest in relation to preeclampsia [24, 25]. The *ADAMTS13* variant c.1852C>G (p.Pro618Ala) is denoted a single nucleotide polymorphism (SNP) because of its reported allele frequency in the population of >1%. It has likewise been found in high prevalence in patients with pregnancy associated TTP [7, 24, 26]. The studied variants and their effect on ADAMTS13 activity and antigen levels in vitro are referred to in Appendix A table A.1.

Laboratory analyses

Blood sampling

Blood sampling was done whenever the subjects entered the HUNT2 study (1995-1997). Serum was separated from whole blood by centrifugation within 2h at the screening site, immediately placed in a refrigerator together with a blood clot or EDTA whole blood, and transported in a cooler within 1-3 days to the HUNT Biobank, where it was stored at -70°C. DNA was extracted from leukocytes from thawed blood clots/EDTA whole blood, using Gentra Puregene Blood Kit (Qiagen Diagnostics GmbH, Hamburg, Germany) and stored at -20°C.

ADAMTS13 genotyping

Genotyping was performed at the HUNT biobank, using TaqMan-based 7900HT Fast Real-Time PCR System (Applied Biosystems, now Thermo Fisher Scientific Corp, MA, USA). Assays for the *ADAMTS13* variants c.3178C>T, c.4143_4144dupA, and c.1852C>G were provided custom made from Applied Biosystems (now Thermo Fisher Scientific). Genotyping success rates were 99.6 % for variant c.1852C>G, 99.1% for variant c.4143_4144dupA and 97.3% for variant c.3178C>T. The genotyping was performed blinded to case-control status.

ADAMTS13 activity

ADAMTS13 activity was assayed in serum samples by the FRETS-VWF73 assay as described by Kokame et al. and Kremer Hovinga et al. [27, 28]. All samples were analysed in duplicates.

Statistics

Categorical variables were described using frequency and percentage. Continuous variables were described using mean ± standard deviation (SD). Means were compared using independent sample t-test, if the variable passed the tests of normality evaluated by histogram, normality Q-Q plot and Shapiro-Wilk's test. Pearson Chi-Square, Fisher exact or Cochran-Armitage (CA) test, where applicable were used to compare genotype results for cases versus controls. Odds ratios (ORs) with their 95% confidence intervals (CI) were calculated to model preeclampsia as the dependent variable against ADAMTS13 genotypes or allele burden in a dominant and an additive genetic model, respectively. Data on ADAMTS13 activity were divided in quartiles, determined by the ADAMTS13 distribution in the control group.

Logistic regression analyses were used to calculate crude and multi-adjusted ORs and their 95% CI to estimate the association between ADAMTS13 activity level and preeclampsia. Adjustments were made for factors related to women's health at the time of participation in the HUNT2 survey (age, body mass index (BMI), serum cholesterol, triglycerides, self-reported parity and number of pregnancies, and socio-economic status determined by level of education, pay insolvency, and dependency of social support), and factors related to preeclampsia risk at the time of pregnancy reported in MBRN (parity, age, previous preeclampsia, multiple pregnancy). A p-value was then calculated using the Chi-Square test for trend for the ADAMTS13 activity.

Haplotypes were predicted from genotype information using the software PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) [29] and association with preeclampsia was calculated by Pearson Chi-Square test.

The statistical analyses were then repeated in a subgroup analysis of women with recurrent preeclampsia, defined as more than one preeclamptic pregnancy.

P-values were two-tailed, and statistical significance level α was set to 0.05 without adjusting for multiple testing.
Statistical analyses were performed using the IBM SPSS Statistics version 19.0 for Windows (IBM Corp, New York, NY, USA) and genetic association study tool set PLINK [29].

Hardy-Weinberg Equilibrium testing

The allelic variants were tested for possible deviations from the Hardy-Weinberg Equilibrium (HWE) in the control group, using the HWE calculator from the Online Encyclopedia for Genetic Epidemiology (http://www.oege.org/software/hardy-weinberg.html). All three sequence variants were found to be in HWE.

Power estimates

An a priori power calculation was performed based upon the following: 1) A pilot study of *ADAMTS13* mutation c.3178C>T in an unselected sample of 250 HUNT2 participants found a prevalence of 2% heterozygous mutation carriers (von Krogh AS et al., in preparation). We considered a difference in prevalence of 6% in women with preeclampsia versus 2% in controls to be of clinical interest, resulting in 89% power to detect such a difference with a sample size of 500 in each group and α=0.05. 2) A study performed by Stepanian et al. [9] on the association between VWF/ADAMTS13 and preeclampsia found an ADAMTS13 activity of 66% (SD 17) in preeclampsia cases versus 78% (SD 16) in controls. A two-sided test with power 90% and α=0.05 would require a sample size of 86 based on these numbers. However, activity analyses were performed at the time of preeclampsia in their study, and we therefore increased the sample size to 100 random cases and 100 random controls. Finally, we analysed ADAMTS13 activity in all carriers of rare variants (both cases and controls) and included them in the sample size. The selection process of subjects included in the ADAMTS13 activity analyses is described in Appendix A figure A.2.

Results

Patients' characteristics

Out of 1000 women, 994 were included in the analyses, after exclusion of one subject due to withdrawal of consent and exclusion of five controls recorded with preeclampsia in the data from MBRN. The clinical characteristics of the study participants are presented in table 1. The age differed significantly between women with preeclampsia compared to controls, due to how cases and controls were defined in the present study. The MBRN has recorded all births in Norway since 1967, and as cases in the present study were defined as women with a diagnosis of preeclampsia recorded in MBRN, only women who had given birth after 1967 were included as cases. Some of the controls, however, had given birth before 1967 and were not recorded in the MBRN. Mother's age at first child or last child did not differ between the two groups. Differences in height, systolic blood pressure, serum cholesterol, and delivery number could be attributed to differences in age. These dissimilarities disappeared when comparing cases with only the controls registered in both the HUNT2 survey and the MBRN (N=500 and N=327, respectively).

Table 1: Baseline characteristics of women with preeclampsia and controls at the time of participation in the HUNT2 survey.

Characteristics	Women with preeclampsia (N=500)	Controls (N=494)	P-value
Age at participation in HUNT2 - years	38.3 ± 10.1	52.0 ± 16.8	<0.001
Age at registration in MBRN - years	24.2 ± 4.7	24.5 ± 5.4	0.37
Height - cm	165.4 ± 5.6	163.4 ± 6.3	<0.001
Weight - kg	74.8 ± 14.5	70.3 ± 12.4	<0.001
Body Mass Index	27.3 ± 5.1	26.3 ± 4.5	0.001
Systolic blood pressure - mmHg	134 ± 19	139 ± 25	0.001
Diastolic blood pressure - mmHg	81 ± 12	80 ± 13	0.40
Cholesterol - mmol/l	5.60 ± 1.11	6.01 ± 1.30	<0.001
HDL cholesterol - mmol/l	1.45 ± 0.38	1.48 ± 0.39	0.30
Delivery number	2.2 ± 1.1	2.6 ± 1.2	<0.001
Number of times pregnant	2.8 ± 1.5	3.1 ± 1.5	0.03
Age at first child	23.8 ± 4.2	23.3 ± 4.1	0.07
Age at last childbirth - years	29.9 ± 4.7	30.4 ± 5.2	0.15
Daily cigarette smoker	113/476 (24%)	135/469 (29%)	0.08
Family history of myocardial infarction < 60 years			
• mother	16 (3.3%)	13 (2.7%)	0.13
• father	70 (14.0%)	49 (9.9%)	0.05

Data are reported as mean ± standard deviation or absolute number and percentages; MBRN, Medical Birth Registry of Norway

ADAMTS13 genotypes

The distribution of genotype and allele frequencies of c.3178C>T, c.4143_4144dupA, and c.1852C>G of *ADAMTS13* is presented in table 2. There were no substantial differences in the genotype and allele frequencies between women with preeclampsia and their controls. For the mutations c.3178C>T and c.4143_4144dupA no homozygous mutation carriers were identified, and the frequencies of heterozygous mutation carriers were low. Using the dominant genetic model, we found no evidence of an increased risk of preeclampsia for heterozygous or homozygous variant genotypes versus homozygous wild-type genotype of the *ADAMTS13* variants examined, i.e. c.3178C>T (OR=0.50, p=0.42), c.4143_4144dupA (OR=4.00, p=0.22), and c.1852C>G (OR=0.92, p=0.62). Similarly, for the additive genetic model (trend in risk with increasing number of variant alleles), we found no clear association: c.3178C>T (χ^2=0.68, p=0.41), c.4143_4144dupA (χ^2=1.80, p=0.18), and c.1852C>G (χ^2=0.06, p=0.81).

Table 2: Genotype distribution of *ADAMTS13* variants.

ADAMTS13 variant		**Cases N**	**Controls N**	**Association**	
				Dominant model	Additive model
c.3178C>T	CC	484	479	χ^2=0.68 p-value=0.41	χ^2=0.68 p-value=0.41
	CT	2	4		
	TT	0	0		
	CT or TT	2	4	OR=0.50 (CI:0.09, 2.71) p-value=0.42	OR=0.50 (CI:0.09, 2.71) p-value=0.42
	c-allele (wild type)	970	962		
	t-allele (variant)	2	4		
c.4143_4144dupA	-/-	490	491	χ^2=1.80 p-value=0.18	χ^2=1.80 p-value=0.18
	-/A	4	1		
	A/A	0	0		
	-/A or A/A	4	1	OR=4.00 (CI:0.45, 35.99) p-value=0.22	OR=4.00 (CI:0.45, 35.99) p-value=0.22
	- allele (wild type)	984	983		
	A-allele (variant)	4	1		
c.1852C>G	CC	412	401	χ^2=0.35 p-value=0.56	χ^2=0.06 p-value=0.81
	CG	81	90		
	GG	5	1		
	CG or GG	86	91	OR=0.92 (CI:0.66, 1.27) p-value=0.62	OR=0.97 (CI:0.72, 1.33) p-value=0.87
	C-allele (wild type)	905	892		
	G-allele (variant)	91	92		

Odds ratio (OR) with confidence interval (CI) for preeclampsia tested for three different *ADAMTS13* variants in a dominant and an additive genetic model. χ^2 and p-values calculated by Fisher exact test or Cochran-Armitage test for the dominant and additive model, respectively

Haplotypes

Out of eight inferred haplotypes, two were common, four were rare (<1%) and two were not observed. The four haplotypes with the highest frequencies are presented in table 3. The CC-haplotype was the most common among both cases and controls (90.3% and 90.3%). No association between rare haplotypes and preeclampsia was observed.

Table 3: Haplotype association in *ADAMTS13.*

ADAMTS13 variant	Haplotypes			
	I	II	III	IV
c.1852C>G (rs28647808)	C	G	C	C
c.3178C>T (rs142572218)	C	C	T	C
c.4143_4144dupA (rs387906343)	-	-	-	A
Frequency in women with PE (958 haplotypes)	865 90.3%	87 9.1%	2 0.2%	4 0.4%
Frequency in Controls (960 haplotypes)	867 90.3%	88 9.2%	3 0.3%	1 0.1%
p-value	1	1	0.66	0.18
Frequency in women with recurrent PE (134 haplotypes)	117 87.3%	17 12.7%	0	0
p-value	0.26	0.20		

Haplotype frequency in absolute number and percentage; P-values calculated by Pearson Chi Squares; PE, preeclampsia. Calculations were made for the whole group and for a subgroup of women with recurrent preeclampsia

ADAMTS13 activity

Tables 4 and 5 display the ADAMTS13 activity in relation to genotype and in relation to preeclampsia status, respectively. The sub-group included in the ADAMTS13 analyses were representative for the whole group of cases and controls in terms of age and number of times pregnant. A total of 302 subjects had ADAMTS13 activity results and were included in the parametric tests after exclusion of two subjects (one case and one control) due to extreme ADAMTS13 values. After exclusion of the two outliers in the positive range, the ADAMTS13 activity results were approximately normally distributed for cases as well as controls and for all genotype groups. The ADAMTS13 activity was lower in c.3178C>T and c.4143_4144dupA mutant allele carriers compared to wild-type, and the differences were statistically significant. For c.1852C>G carriers heterozygous allele carriers had significantly lower activity compared to wild-type. Few women were homozygous for c.1852C>G, their mean ADAMTS13 activity was lower than wild-type, but the differences were not statistically significant.

We found no significant association between ADAMTS13 activity in quartiles and the risk of preeclampsia. Adjustment for preeclampsia risk factors did not alter the results.

Forty-two women in the preeclampsia group had not been pregnant before participating in the HUNT2 study, i.e. had their sample drawn before the outcome preeclampsia. Their ADAMTS13 activity (mean 156 ± 34) did not differ from that of the women who had been pregnant before participating in the HUNT2 study.

The subgroup of women with recurrent preeclampsia comprised 67 subjects, and no association to ADAMTS13 haplotypes, individual variants or ADAMTS13 activity was found (data shown only for haplotypes, table 3).

Table 4: ADAMTS13 activity in relation to genotype.

	Genotype variant (N)		% activity mean (SD)	p-value
c.3178C>T	C/C	(293)	153 (33)	
	C/T	(6)	101 (7)	<0.0001
c.4143_4144dupA	-/-	(296)	154 (33)	
	-/A	(5)	83 (20)	<0.0001
c.1852C>G	C/C	(179)	157 (34)	
	C/G	(117)	147 (33)	=0.01
	G/G	(6)	137 (41)	=0.17

Differences in mean ADAMTS13 activity for different genotypes, calculated by independent samples t-test

Table 5: ADAMTS13 activity in women with preeclampsia and controls.

	Preeclamptic women	Controls	Chi-square test for trend	Odds ratio	Adjusted Odds ratio[1]	Adjusted Odds ratio[2]
ADAMTS13 activity %	Mean ± SD 155 ± 35	Mean ± SD 150 ± 33				
	Number	Number				
1.quartile <128	30	35	$X^2 = 1.50$	OR 0.64 (0.33, 1.23)	OR 0.20 (0.02, 1.99)	OR 0.84 (0.39, 1.79)
2.quartile 128-150	44	37		OR 0.73 (0.38, 1.42)	OR 0.33 (0.05, 2.25)	OR 0.70 (0.31, 1.59)
3.quartile 151-172	41	35	p= 0.22	OR 0.72 (0.37, 1.39)	OR 0.30 (0.05, 1.88)	OR 0.92 (0.43, 1.99)
4.quartile >172	47	35		reference	reference	reference
Total	162	142				

Mean is ADAMTS13 activity in percent of normal plasma ± standard deviation (SD). Means were not statistically different for women with PE and controls (p-value 0.26).

OR indicates unadjusted Odds ratio (OR) (with 95% confidence intervals) for ADAMTS13 activity in the lower quartiles compared to the highest quartile; Odds ratio[1] adjustment for age, BMI, cholesterol, triglycerides, parity and number of pregnancies, level of education, dependency on social support, and pay insolvency at time of participation in the HUNT2 survey; Odds ratio[2] adjustment for age, parity, previous preeclampsia, and multiple pregnancy at the time of pregnancy

Discussion:

In this study of the association between three functional variants in the *ADAMTS13* gene and the risk of preeclampsia, we found no increased risk in women carrying the mutations c.3178C>T, c.4143_4144dupA, and the SNP c.1852C>G.

Allelic variants in the present study were chosen not as tag SNPs for the study of a general association between *ADAMTS13* gene polymorphisms and preeclampsia, but because of their reported prevalence in women with pregnancy-associated hereditary TTP in a direct hypothesis testing manner. Two of the variants are very rare (c.3178C>T and c.4143_4144dupA), but have a high functional impact on the protease activity. This has been demonstrated in previous studies and was also displayed in the significantly lower ADAMTS13 activity in serum of women carrying these allelic variants in the present study. A large effect size on the outcome could be anticipated from the functional impact of the variants. However, no association to preeclampsia was found in the present study, suggesting there is no association of *clinical interest* for the three studied *ADAMTS13* variants.

Variants in the *ADAMTS13* gene have been investigated in genetic association studies of various disorders of thromboembolism and endothelial inflammation [10, 30-33] , but never in preeclampsia. The *ADAMTS13* variant c.1852C>G has been studied in association with risk of death or cardiac death among chronic coronary heart disease patients [30]. No association was found for this SNP, while variant c.2699C>T showed increased risk. The variant c.1852C>G was also studied in relation to deep venous thrombosis (DVT), with no association observed [34]. The rarer variants c.3178C>T and c.4143_4144dupA have not previously been investigated in association studies.

An area of interest has been the influence of other co-existing *ADAMTS13* variants on the metalloprotease activity in plasma. Plaimauer et al. showed that the presence of c.2195C>T(p.Ala732Val) together with c.1852C>G (p.Pro732Ala) further decreased antigen and activity level of ADAMTS13 in constructs, whereas c.19C>T (p.Arg7Trp, rs34024143) and c.1342C>G (p.Gln448Glu, rs2301612) together with c.1852G>C increased secretion of ADAMTS13, although not until full recovery of activity [35]. Such influence of co-existing SNPs could be of relevance also in our

study. Instead of investigating further *ADAMTS13* variants, we inferred haplotypes to look at possible mutual interplay of the three variants under study. Nevertheless, looking at haplotypes rather than at single variants, no association to preeclampsia was observed.

We found no association between ADAMTS13 activity and the outcome preeclampsia in our study population. This supports lack of association between functional *ADAMTS13* variants and preeclampsia, but diverges from the findings of Stepanian et al. in their study on VWF/ADAMTS13 interplay in preeclampsia [9]. They found increased VWF antigen levels and decreased ADAMTS13 activity and antigen with strong association to preeclampsia, indicating a misbalance favouring microvascular thrombosis. An important difference between their study and the present is that they analysed ADAMTS13 activity at the time of diagnosis of preeclampsia. In our study the time of blood sampling was not related to the time of the event, ranging from 16 years before until 28 years after pregnancy, which eliminated any potential effect of preeclampsia on the ADAMTS13 levels. ADAMTS13 activity varies with age and in different physiological and pathological conditions. Lower levels are found in new-borns and in older people, and in the second and third trimester of normal pregnancies [36, 37]. Alpoim and colleagues found decreased ADAMTS13 levels in preeclamptic women compared to normotensive pregnant women[38], while in other studies a significant ADAMTS13 reduction was limited to the rarer pregnancy complication HELLP syndrome (Haemolysis Elevated Liver enzymes, Low Platelets)[39]. Others again have found normal ADAMTS13 levels but increased VWF in preeclampsia[40]. This shift of balance between VWF and ADAMTS13 to a more pro-thrombotic state is seen in various situations of inflammation and endothelial cell activation. The processes that regulate ADAMTS13 activity in these situations are not fully understood, but may include consumption of ADAMTS13 (due to adherence of ADAMTS13 to VWF attached to the endothelial cells), different kinetics of VWF versus ADAMTS13 release after synthesis, or proteolytic alteration of the VWF cleaving protease.

We designed our study to eliminate a possible effect of pregnancy and preeclampsia on ADAMTS13 activity. We performed a subgroup analysis among the women with preeclampsia who participated in the HUNT2 study before their first pregnancy and found no difference in mean ADAMTS13 activity, compared to women with blood drawn after the event (preeclamptic pregnancy). This underscores that ADAMTS13 results in the present study were independent of the outcome. Stepanian et al. investigated inflammatory cytokines and CRP as well as ADAMTS13 activity in their study [9]. They suggest that the decrease in ADAMTS13 activity found in preeclampsia was at least partly dependent on increased mediators of inflammation (IL-6, CRP), as adjustment for IL-6 and CRP suppressed the observed association between ADAMTS13 activity and preeclampsia. This indicates that inflammation may be an initiating event to the VWF/ADAMTS 13 misbalance, and that an observed association is a marker of the event rather than a causal correlation.

Mean ADAMTS13 activity was higher than expected in the present study, and this applied for all samples. Heterozygous carriers of variants c.3178C>T and c.4143_4144dupA had an activity of 54-65% of that of homozygous wild-type carriers, somewhat higher than expected. The high values of ADAMTS13 activity can not solely be explained by the fact that we used serum samples, which theoretically could give slightly higher ADAMTS13 values. Serum and citrated plasma samples have previously been found comparable in activity assays [41]. The reason remains unclear, but could be related to population differences in ADAMTS13 activity or factors related to sample storage.

A potential limitation of the present study is the difference in age between women with preeclampsia and their controls, cases being significantly younger than controls at the time of participation in the HUNT2 survey. ADAMTS13 activity decreases with age, and could abolish a difference between cases and controls. However, adjustment for age in the multivariate analysis gave the same result. Since some of the women in the control group had given birth before 1967, and these births were not registered in the MBRN, some of these women could in fact be cases. This would, however, only account for a small proportion of the women (about 4%, statistically). No differences in ADAMTS13 activity was found when comparing women with their first delivery before 1967, with women with their first delivery after 1967. Additionally, when we performed analyses restricted to cases and controls with data registered in the MBRN, no association between ADAMTS13 activity and preeclampsia was observed.

Frequencies of mutations c.3178C>T and c.4143_4144dupA were lower than anticipated from a pilot study on *ADAMTS13* mutations in the HUNT2 study (von Krogh AS et al., in preparation). The study is therefore underpowered in order to detect a small increase in risk for these two variants. The minor allele frequency of variant c.1852C>G (9%), however, was in perfect accordance with the frequency in a European population from the 1000 genomes project (www.1000genomes.org).

Studying the genetic contribution in preeclampsia faces certain challenges. A familial predisposition suggests a genetic component of the disease, and twin studies have estimated this heritability to be approximately 55% [42]. Results from studies of possible candidate genes are diverging. The areas of focus for these studies have been pathophysiological pathways involved in thrombophilia, vasoactive proteins, hypo-fibrinolysis, oxidative stress, lipid metabolism, endothelial injury and immunogenetics [43]. A major challenge in many of these studies is the selection of cases and controls. The fact that all cases in the present study had a valid diagnosis of preeclampsia strengthens the validity of our results. The diagnostic validity of the diagnosis of preeclampsia was assessed by examining medical records according to the definition of preeclampsia used by the MBRN. Our study was, however, not powered to investigate a small effect size, nor a possible effect in certain subtypes of preeclampsia. Since preeclampsia is a common and complex disease, observed to cluster within families, it is possible that the genetic contribution may vary in certain subtypes of the disease and from family to family. In the search of rare variants with large effect sizes a study of family-based cohorts including cases of preeclampsia would therefore be of interest.

To conclude, we found no association between *ADAMTS13* gene variants c.3178C>T, c.4143_4144dupA, and c.1852C>G and preeclampsia in this large case- control study from the HUNT2 study. Furthermore, no association between ADAMTS13 activity and preeclampsia was found when investigated unrelated in time to the event.

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***Highlights (for review)**

Highlights

- We study functional *ADAMTS13* variants in relation to preeclampsia
- This is done in a large, nested case-control study
- We find no association to preeclampsia (PE)
- Likewise, no association is found between ADAMTS13 activity and PE